Bioorganic & Medicinal Chemistry Letters xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

# Synthesis of novel steroidal agonists, partial agonists, and antagonists for the glucocorticoid receptor

Zhuang Jin [a], Hua Lin [a], Sathish Srinivasan [b], Jerome C. Nwachukwu [b], Nelson Bruno [b], Patrick R. Griffin [a], Kendall W. Nettles [b,*], Theodore M. Kamenecka [a,*]

[a] Department of Molecular Therapeutics, The Scripps Research Institute, Jupiter, FL 33458, USA
[b] Department of Cancer Biology, The Scripps Research Institute, Jupiter, FL 33458, USA

ARTICLE INFO

*Article history:*
Received 29 August 2016
Revised 2 November 2016
Accepted 3 November 2016
Available online xxxx

*Keywords:*
Agonist
Partial agonist
Antagonist
Glucocorticoid receptor

ABSTRACT

Adverse effects of glucocorticoids could be limited by developing new compounds that selectively modulate anti-inflammatory activity of the glucocorticoid receptor (GR). We have synthesized a novel series of steroidal GR ligands, including potent agonists, partial agonists and antagonists with a wide range of effects on inhibiting secretion of interleukin-6. Some of these new ligands were designed to directly impact conformational stability of helix-12, in the GR ligand-binding domain (LBD). These compounds modulated GR activity and glucocorticoid-induced gene expression in a manner that was inversely correlated to the degree of inflammatory response. In contrast, compounds designed to directly modulate LBD epitopes outside helix-12, led to dissociated levels of GR-mediated gene expression and inflammatory response. Therefore, these new series of compounds and their derivatives will be useful to dissect the ligand-dependent features of GR signaling specificity.



Glucocorticoids have a profound impact on human physiology, including effects on energy metabolism, bone endocrinology, and immune, stress and inflammatory responses. Glucocorticoids exert their effect via binding to and modulating the activity of the glucocorticoid receptor (GR), a member of the nuclear receptor superfamily of ligand-regulated transcription factors. GR agonists such as dexamethasone[1,2] and fluorocortivazol[3,4] (Fig. 1), are the most widely prescribed drugs in the United States, largely because of their anti-inflammatory effects in diseases such as asthma, rheumatoid arthritis and inflammatory bowel disease. However, detrimental side effects such as diabetes, muscle wasting and osteoporosis limit their therapeutic use. GR antagonists also have therapeutic value, for example as anti-diabetic agents[5–14] and potentially in castrate-resistant prostate cancer.[15] The search for selective GR modulators that retain potent anti-inflammatory properties without adverse side effects has led to clinical trials, but not available drugs.

Efforts to improve GR modulators are currently hampered by poor understanding of the biophysical principles that guide GR signaling specificity. To regulate transcription, GR utilizes all of its modular domains, including an intrinsically disordered, amino-terminal domain that contains a coactivator-binding site called activation function-1 (AF-1), a central zinc-finger DNA-binding domain, and a carboxyl-terminal LBD that contains a ligand-regulated, coactivator-binding surface called AF-2. In the canonical signaling pathway, GR translocates to the nucleus, where it binds directly to specific DNA sequences known as glucocorticoid-response elements (GREs), which are typically located in the regulatory regions of its target genes. A distinct non-canonical signaling pathway, where GR is tethered to DNA by transcription factors such as AP-1 and NF-κB, has also been described for a subset of target genes that lack classic GREs, including inflammatory genes.[16] At target genes, GR recruits a set of nuclear receptor coactivators and corepressors[17], which collectively provide all of the enzymatic activities required to control RNA polymerase II and other components of the general transcription machinery.

Selective modulation of GR signaling is initiated by the ligand, which binds inside a hydrophobic pocket in the ligand-binding

*Abbreviations:* AF-1, activation function-1; AF-2, activation function-2; AP-1, activator protein 1; dppp, 1,3-bis(diphenylphosphino)propane; DEX, dexamethasone; DIEA, *N,N*-diisopropylethylamine; FKBP5, FK506-binding protein 5; GR, glucocorticoid receptor; GRE, glucocorticoid-response element; HATU, 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate; IL-6, Interleukin-6; LBD, ligand-binding domain; LPS, lipopolysaccharide; MMTV, mouse mammary tumor virus; NF-κB, nuclear factor-kappa B; Pdk4, pyruvate dehydrogenase lipoamide kinase isozyme 4; qPCR, quantitative real time polymerase chain reaction.

* Corresponding authors.
*E-mail address:* kameneck@scripps.edu (T.M. Kamenecka).

http://dx.doi.org/10.1016/j.bmcl.2016.11.007
0960-894X/

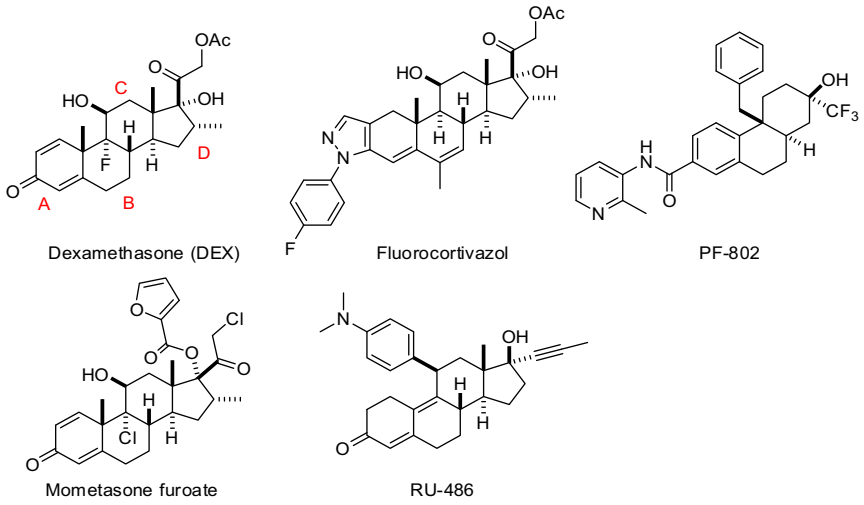
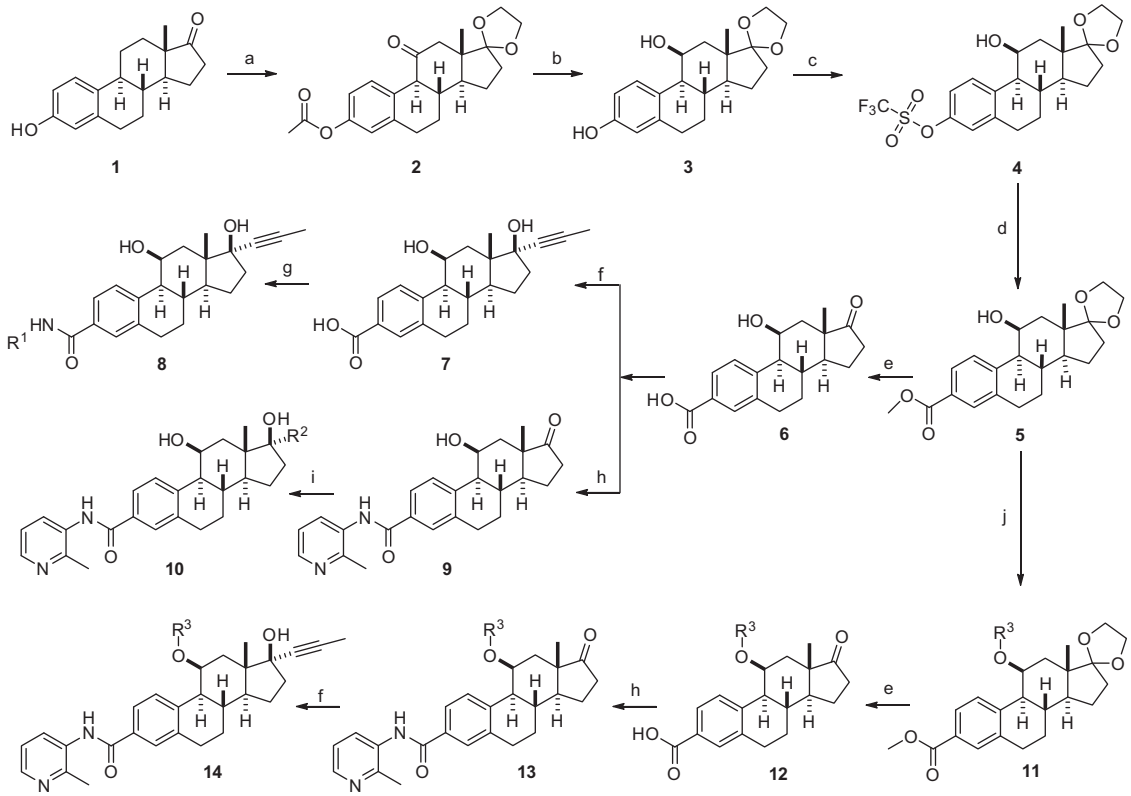



**Fig. 1.** Examples of GR ligands.

domain (LBD). In the active conformation, helix 12 in the LBD docks across helix 3 and helix 11 to form one side of the AF-2 coactivator-binding surface.[18] Antagonists such as RU-486 contain a protruding side group that physically destabilizes the active conformation, thereby blocking formation of an intact AF-2 surface and inhibiting coactivator recruitment. Structures of the LBD in complex with different selective GR modulators rarely show obvious differences in helix 12 positioning or overall architecture[19,20], suggesting that the conformational changes that drive GR signaling specificity are quite subtle.

Most synthetic GR ligands can be compared to a steroid such as dexamethasone, where the four core rings are numbered A-D (Fig. 1). However, seco-steroids and non-steroids (i.e. Mapracorat) have more recently been pursued as well.[21,22] Previous reports on ligand-bound GR complexes suggest at least three key sites for modification of the steroidal scaffold. At the first site, there is typically an A-ring ketone that engages in hydrogen bonding for potency and selectivity. In the crystal structure of GR bound to deacylcortivazol, this ketone was replaced with a phenyl pyrazole group, which opened up a new pocket to access a solvent channel



**Scheme 1.** Synthetic Pathway for GR Receptor Modulators. Reaction Conditions: (a) Ref. 23, 5 steps; (b) $NaBH_4$,THF:MeOH (1:1); (c) *N*-Phenyl-bis(trifluoromethanesulfonimide), $Et_3N$, $CH_2Cl_2$; (d) $Pd(OAc)_2$, dppp, $Et_3N$, MeOH, CO, DMF; (e) LiOH, THF: $H_2O$; aqueous HCl (20%); (f) 1-propynyl-MgBr, THF; (g) HATU, DIEA, $R^1NH_2$, DMF; (h) 3-aminopicoline, HATU, $Et_3N$; (i) $R^2M$ (M = MgBr or Li), THF; (j) $R^3X$ (X = Cl or Br), NaH, DMF.

Please cite this article in press as: Jin Z., et al. Bioorg. Med. Chem. Lett. (2016), http://dx.doi.org/10.1016/j.bmcl.2016.11.007